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Original Research

Screening of total phenolic, antiradical and antioxidant activities of *Wiedemannia orientalis*

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Abstract: In this study, it was aimed to investigate total phenolic, antioxidant and antiradical activities of water and ethanolic extracts of the *Wiedemannia orientalis (W. orientalis)*. In order to investigate the antiradical capacities, DPPH and ABTS radical scavenging activates were employed in the evaluation process. For the evaluation of antioxidant capacities, activities of phosphomolybdenum assay, reducing power and metal chelating were investigated. In the evaluations, the amounts of total phenolics were determined to be 11.95±0.02-17.27±0.09 mg Gallic acid equivalents (GAE)/g in *W. orientalis* extracts, respectively. Additionally, the amount of reducing power and total antioxidant capacity of leaf ethanol extract of *W. orientalis* were determined to be higher compared to the other extracts of *W. orientalis*. While the highest activity was observed in flower ethanol extract on the DPPH radical scavenging activity, flower water extract demonstrated better results in metal chelating activity. In the ABTS radical chelating activity, no significant differences were observed. As a result, it was suggested in our study that extracts of *W. orientalis* could be regarded as a natural and alternative source in pharmacology and medicine and food sectors. Such results can be put to use in pharmaceutical formulations and may lead to the developments of new human drugs from this medicinal plant.

Key words: *Wiedemannia orientalis*; Antiradical activity; Antioxidant activity; Phenolic.

Introduction

A major emphasis was placed on natural products and healthy foods in terms of improving overall well-being, preventing diseases and incorporating substances that promote health into diets as natural food additives (1-3). Therefore, the attention was focused on developing and isolating natural antioxidants out of botanical sources, particularly plants. Plant phenolics are the primary source of antioxidants. Additionally, they are multifunctional and can act as metal chelators, singlet oxygen quenchers and reducing agents (free radical terminators) (4-6).

In the flora of Turkey, the genus *Wiedemannia* (Lamiaceae) is represented by two species (W. orientalis and W. multifida). *W. orientalis* Fisch. & Mey. is an endemic species and found commonly in Anatolia (7). The Lamiaceae family contains a number of plants that are famous for their powerful antimicrobial and antioxidant properties. Plants that belong to the Lamiaceae family are abundant in polyphenolic compounds with more than 160 polyphenols identified, some of which are unique to the genus (8, 9). While many plants belonging to Lamiaceae and their derivatives were investigated in detail as antimicrobial and antioxidant compounds (8, 10-12), only two studies were conducted regarding the chemical constituents of *W. orientalis* (7, 13). Nevertheless, the antimicrobial and antioxidant effects of *W. multifida* and *W. orientalis* were not investigated until now.

In the current study, it was aimed to designate the total phenolic compounds and, antiradical and antioxidant activities of ethanolic and water extracts of flowers and leaves of *W. orientalis*, which is an endemic plant in Turkey.

Materials and Methods

Plant Material and Extraction Process

In this study, antioxidant activities and radical scavenging capacities in the extracts of *W. orientalis* were investigated. The leaves and flowers of *W. orientalis* were gathered on June 2009 at the 1250-m high Karga Mountain (around Ortaçalı Village, southern slopes) located in the center of Elazig. The identification of the gathered material was carried out at the Herbarium in Firat University, Elazig. The fresh plant material was washed with tap water, dried, and then chopped into smaller pieces. Then, the pieces were dried under shade and reduced to coarse powders using a mortar and a pestle. In order to obtain the ethanol extract of *W. orientalis*, the powdered plant material (10 g) was extracted by using a Soxhlet-type extractor with 100 mL ethanol at 60 °C for 6 h. Then, the extracts were filtered through a Buckner funnel and Whatman No. 1 filter paper. For the water extracts, 100 g plant sample was held in 200 mL boiling water for 10 min. Each filtrate was concentrated to dryness under reduced pressure at 40 °C by using a rotary evaporator. All extracts were resuspended in dimethyl sulfoxide (DMSO-d6) in order to obtain 50 mg/mL stock solution.

Determination of total phenolics

In the estimation of the total phenolic contents of plant extracts, a colorimetric assay, which was based on

the procedures of Singleton & Rossi, was used (14). In short, 1 ml of sample was combined with 1 mL of Folin and Ciocalteu's phenol reagent. After three minutes, 1 mL of saturated sodium carbonate solution was included in the mixture and then the mixture was adjusted to 10 mL by using distilled water. For 90 minutes, the reaction was kept in dark. Following this process, the absorbance was measured at 765 nm by utilizing a Shimadzu 1240 spectrophotometer. Additionally, gallic acid was employed in the creation of the standard curve. The estimation of the phenolic content was conducted out in triplicates. The acquired results were mean values and expressed as mg of gallic acid equivalents (GAE)/g of the dry extract.

Reducing power

The reducing power was determined according to the method of Oyaizu (15). Various concentrations of *W. orientalis* water and ethanolic extracts (2.5 mL) were combined with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Following this procedure, the obtained mixture was incubated at 50 C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid (w/v) were included and the mixture was subjected to centrifuging at 650 rpm for 10 minutes. The upper layer (5 mL) was mixed with 5 ml of deionized water and 1 mL of 0.1% of ferric chloride. Then, the absorbance was read at 700 nm: higher absorbance implies higher reducing power. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

Metal chelating activity

The chelating of ferrous ions by the *W. orientalis* extracts and standards were estimated by adopting the method of Dinis et al (16). Shortly, the extracts (50, 100 and 250 µg/mL) were included in a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by adding 5 mM ferrozine (0.2 mL) and the obtained mixture was shaken vigorously and kept at room temperature for 10 min. Then, the absorbance of the solution was read spectrophotometrically at 562 nm.

Determination of antioxidant activity

Total antioxidant activity (Phosphomolybdenum assay)

The total antioxidant activities of the plant extracts were calculated by using the phosphomolybdenum method described by Prieto *et al* (17). In short, 0.3 mL of a 1 mg/mL extract solution in ethanol was combined with 2.7 mL phosphomolybdenum reagent in capped test tubes. The incubation was then conducted for 90 min in a water bath at 95 °C. Following the cooling down to room temperature, the absorbance of the solutions was read by using a spectrophotometer (Shimadzu 1240 spectrophotometer) at 695 nm against a blank (0.3 mL ethanol without plant extract). The antioxidant activity was calculated by using a standard curve with ascorbic acid solutions as the standard. The mean values of three readings were used and the reducing capacities of the extracts were expressed as mg of ascorbic acid equivalents (AAE)/g extract.

DPPH· radical scavenging capacity

2,2-diphenyl-1-picryl-hydrazyl (DPPH·) free radical-scavenging activity of water and ethanol extracts from *W. orientalis* were evaluated by the method of Blois (1958) (18) with a slight modification by Gülçin (2010) (19). Shortly, different concentrations (50, 100, and 250 µg/mL) of water extract or ethanol extract from *W. orientalis* were prepared and volume adjusted to 3 mL with ethanol. One milliliter of 0.1 mM ethanolic DPPH solution was included in the samples. Then, the samples were vortexed and incubated in dark at room temperature for 30 min. Following this process, the absorbance was read at 517 nm against blank samples. The decreased absorbance of the sample indicates DPPH· free radical scavenging capability.

ABTS·+ radical scavenging capacity

The spectrophotometric analysis of ABTS·+ radical scavenging capacity was determined according to the method of Re et al. (20). The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 16 h at room temperature in the dark until the reaction was complete and the absorbance was stable. The ABTS·+ solution was diluted with ethanol to an absorbance of 0.750±0.025 at 734 nm for measurement (Shimadzu 1240 spectrophotometer).

Results

In this study, the phenolic compositions, antiradical and antioxidant activities of *W. orientalis* extracts were investigated. The total phenolic contents of *W. orientalis* extracts were demonstrated in Table 1. The total phenolic contents of extracts determined by using the Folin-Ciocalteau method were expressed as gallic acid equivalents. As it can be seen in Table 1, the total phenolics contents of *W. orientalis* leaf [(17.27±0.09) mg GAE/g extract] extract were observed to be higher compared to other extracts of *W. orientalis*.

The total antioxidant activities of the extracts calculated by phosphomolybdenum assay were presented in Table 1. Both extracts of *W. orientalis* leaves and flowers demonstrated powerful total antioxidant activities. Total antioxidant activities of ethanol and water *W. orientalis* leaves and flowers were determined to be (142.12±0.52) mg, (139.41±0.47) mg, (113.33±0.3) mg

Table 1. Total phenolic content (mg GAE/g extract), total antioxidant activity (mg AAE/g extract) of *W. orientalis* extracts.

Extract (mg/mL)	Experimental	
	Total phenolic content	Total antioxidant activity
Leaf ethanol extract	17.27±0.09	142.12±0.52
Leaf water extract	15.89±0.01	139.41±0.47
Flower ethanol extract	12.54±0.04	113.33±0.3
Flower water extract	11.95±0.02	127.11±0.59

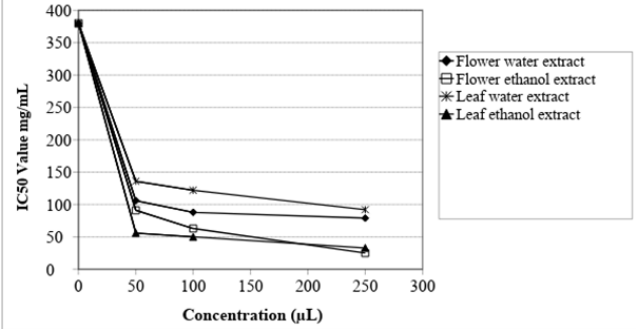

Figure 1. DPPH free radical scavenging activity assay of *W. orientalis* extracts.

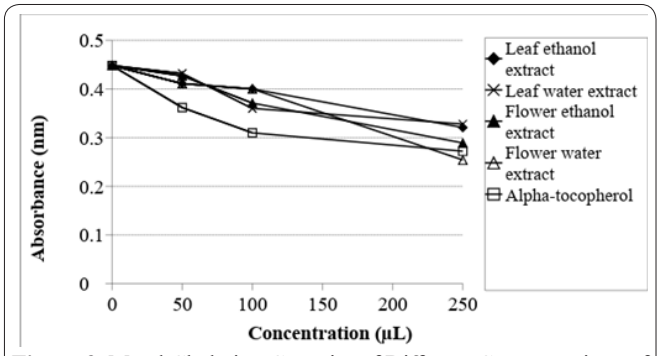

Figure 3. Metal Chelating Capacity of Different Concentrations of Extracts of *W. orientalis* (mg/mL).

and (127.11±0.59) mg ascorbic acid equivalent (AAE)/g dry extract, respectively.

DPPH molecule, which includes a stable free radical, was commonly used to assess the radical scavenging ability of antioxidants. Free radical scavenging activities of the extracts investigated in the DPPH assay were demonstrated in Figure 1.

W. orientalis extracts demonstrated potent free radical scavenging activities. Free radical scavenging activities of the extracts demonstrated an increase with increasing extract concentration (Figure 1). In all of the tested concentrations, *W. orientalis* flower ethanol extract demonstrated considerably powerful activity compared to the other extracts of *W. orientalis*.

Extracts of *W. orientalis* and standards demonstrated high $ABTS^{\cdot}$ radical scavenging activities at 100 µg/mL. At this concentration, the scavenging activities' inhibition values in percentages were 90.5, 90.6, 90.5, 90.6 and 96.9% for α-tocopherol, ethanol and water extracts of *W. orientalis* leaves and flowers, respectively.

Reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Just as the antioxidant activity, the reducing power of *W. orientalis* extracts were observed to be increased with increasing amount of the sample. The reducing power of water and ethanol extracts of *W. orientalis* and the standard compound were ordered as (Figure 2): Leaf ethanol extract>Flower water extract >α-tocopherol>Flower ethanol extract >Leaf water extract.

As it was demonstrated in Figure 3, the formation of the Fe^{2+}-ferrozine complex is incomplete in the presence of the extracts of *W. orientalis*. This situation indicates that the extracts of *W. orientalis* chelate with the iron. The absorbance of Fe^{2+}-ferrozine complex decreased linearly based on the dose (from 50 to 250 µg/mL). The highest metal chelating activity was observed in the flower water extract of *W. orientalis* in 250 µg concentration. The data in Figure 3 suggested that *W. orientalis* extracts had a notable capacity for iron binding. This indicated that their action as a peroxidation protector might be related to its iron binding capacity.

Table 2. $ABTS^{\cdot}$ Radical Scavenging Capacity of Extracts of *W. orientalis.*

Extracts (100 µg/mL)	ABTS assay (%)Inhibition
W. orientalis-leaf ethanol extract	90.5
W. orientalis-leaf water extract	89.6
W. orientalis-flower ethanol extract	92.5
W. orientalis-flower water extract	85.6
α-tocopherol	96.9

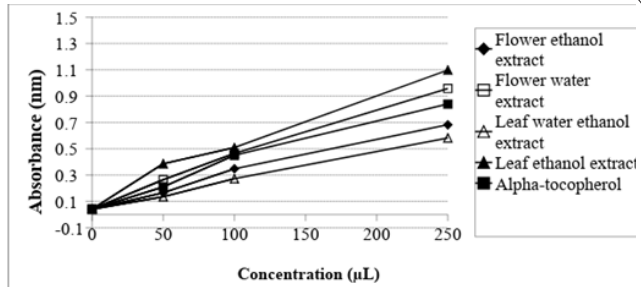

Figure 2. Reducing Power Capacity of Different Concentrations of Extracts of *W. orientalis* (mg/mL).

Discussion

In the through surveying of the literature, no references concerning the total phenolic content, antioxidant and antimicrobial activities of *Wiedemannia* species were found. This study is also the second study regarding the antioxidant and antiradical properties of *W. orientalis.*

In a study conducted by Albayrak et al., the phenolic compositions, antimicrobial and antioxidant activities of *W. multifida* and *W. orientalis* extracts were investigated. The percentage yields of methanolic extracts of W. multifida and W. orientalis were determined to be 20.0% and 24.0%, respectively. The total phenolic contents of W. multifida and W. orientalis extracts, which were calculated by using the Folin-Ciocalteau method, was expressed as gallic acid equivalents. The total phenolics contents of *W. multifida* [(22.45±0.60) mg GAE/g extract] extract were observed to be higher compared to the extracts of *W. orientalis* [(9.53±0.00) mg GAE/g extract] (21). These results were found to be similar compared to our results. In this study, the total phenolic contents of the extracts were determined to be between 17.27-11.95. The lowest value was determined to be in the W.orientalis' flower water extract. In the same study, the total antioxidant activities of the extracts were calculated by the phosphomolybdenum assay. Both of the extracts demonstrated powerful total antioxidant activities. The total antioxidant activities of *W. multifida* and *W. orientalis* were determined as (181.63±0.50) mg and (127.69±0.50) mg ascorbic acid equivalent (AAE)/g dry extract, respectively (21). In this study, the total antioxidant activities for leaf ethanol extract flower water extract, flower ethanol extract and leaf ethanol extract were determined to be 142.12±0.52, 139.41±0.47, 127.11±0.59, 113.33±0.3 mg ascorbic acid equivalent

(AAE)/g dry extract, respectively. DPPH molecule, which contains a stable free radical, was commonly employed to determine the radical scavenging ability of antioxidants. In the study conducted by Albayrak, free radical scavenging activities of the extracts were examined in the DPPH assay. *E*xtracts *of W. multifida* and *W. orientalis* demonstrated potent free radical scavenging activities. Free radical scavenging activities of both of the extracts were observed to be increased with increasing extract concentration. In all of the tested concentrations, *W. multifida* extract demonstrated a considerably stronger activity compared to the *W. orientalis* extract (21). According to the DPPH results obtained in this study, IC_{50} values were calculated and it was determined that the radical scavenging activities of the extract increased with increasing extract concentration. In the examination of the results according to the highest concentration (250 µL), it was determined that *W. orientalis* flower ethanol (IC_{50} 25 mg/mL) extract had the highest purifying capacity. Additionally, in the examination of the purifying capacity results of $ABTS^{+}$, which is a free radical, it was determined that all extract demonstrated excellent activities, however, the highest capacity was that of *W. orientalis* flower ethanol extract (92.5%). In the examination of the metal chelating results, it was determined that the highest chelating activity was that of *W. orientalis* flower water extract and according to the reducing power capacity results, the highest activity was that of *W. orientalis* leaf ethanol extract.

Several studies regarding the antioxidant and antimicrobial activities of various species belonging to *Lamiaceae* were conducted. In one study, Salah *et al.* reported that *Salvia aegyptiaca* (IC_{50} 43.6 mg/mL), *Salvia verbenaca* (IC_{50} 86.9 mg/mL) and *Salvia argentea* (IC_{50} 374.4 mg/mL) extracts demonstrated antioxidant activities in DPPH assay (8). The values observed in the study were lower compared to the tested *Wiedemannia* species. Sokmen *et al.* investigated the antioxidant activity of the polar subfraction of the methanol extract of *Thymus spathulifolius* and they reported in their study that it was able to decrease the stable free radical DPPH with an IC_{50} of (16.15±0.50) µg/mL. This result was also was lower compared to the tested *Wiedemannia* species (10). Turkoglu *et al.* reported that *Salvia russelli* extracts demonstrated high antioxidant activities in DPPH radical scavenging (0.045-0.12 nm), metal chelating (0.184-0.36 nm), ABTS radical scavenging (89.5-90.5%) and reducing power (0.572-0.906 nm) (22). Additionally, Turkoglu *et al*. reported in their study that *Teucrium parviflorum* extracts demonstrated powerful antioxidant activities. This reported antioxidant property was based on the concentration and it increased with the increased amount of the sample. Furthermore, total phenolic compounds in the extracts of T. parviflorum were designated as pyrocatechol equivalents (23). In another study, *Ajuga chamaepitys* extracts demonstrated powerful antioxidant activities such as DPPH radical scavenging (0.087-0.195 nm), metal chelating (0.136-0.199 nm), ABTS radical scavenging (52.4-90.6%) and reducing power (0.241-0.621 nm) (24).

To our knowledge, only two reports about the chemical constituents of *W. orientalis* were reported (7, 13). In the report published by Başer *et al.*, water distilled essential oil from fresh aerial parts of *Wiedemannia orientalis* was investigated by GC and GC-MS, and 31 compounds were identified as germacrene D (38.94%), geijerene (14.60%), and pregeijerene (12.90%) being the major constituents (13). In the other study, five iridoid glycosides, ipolamiide, lamiide, ipolamiidoside, 6β-hydroxyipolamiide, and 5-hydroxy-8-epi-loganin; 5 flavonoid glycosides, luteolin 5-O-β- glucopyranoside, apigenin 7-O-β-glucopyranoside, isorhamnetin, apigenin 7-O-(6β-O-trans-p-coumaroyl) β-glucopyranoside3-O-rutinoside, quercetin 3-O-rutinoside; and a phenylethanoid glycoside, acteoside (verbascoside) were isolated and identified from the aerial parts of *W. orientalis* (7).

Isolated compounds from Wiedemannia orientalis demonstrate different activities. Quercetin 3-O-rutinoside is known to possess an antioxidant activity (25). Lamiide demonstrated an anti-inflammatory activity and inhibited lipid peroxidation (26). Ipolamiide demonstrated an anti-inflammatory activity (27). Ipolamiidoside is reported to have an antiviral activity (28). Acteoside was demonstrated to possess various activities such as anti-inflammatory (27), antioxidant (29), antimutagenic (29), anticarcinogenic (29), and neuroprotective effects (30). Consequently, Wiedemannia orientalis can be a good source for various activities.

Finally, *W. orientalis* extracts showed powerful antioxidant activities. In this way, this study concluded that the water and the ethanolic extracts of *W. orientalis* could be potential sources of natural antioxidants. This study forms the second study concerning *W. orientalis*' antioxidant and antiradical activities. After this monitoring experiment, future studies should be carried out to isolate and identify individual phenolic compounds and identify the antioxidant and antiradical activities in a more detailed way as *in vivo*. The indicated plant extracts are favorable in the pharmaceutical industry as antioxidant and antimicrobial agents acquired from natural resources, which can replace the synthetic agents. However, studies investigating the toxicity of the plant extracts should be conducted in order to establish the safe dosage for humans. The current study symbolizes a good start for other potential properties of plant extracts in living humans. Additionally, because the plants in consideration originate in Turkey, the process of acquiring biological agents from natural sources might cost lower than using synthetic or other imported agents. Researchers could conduct studies on these particular plants by purifying the active components of the plants.

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